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STIRBAR MICROEXTRACTION FOR DETECTION OF CW AGENTS IN LIQUID MATRICES

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This work covers the use of stirbar microextraction for the detection of GB, HD, and VX from liquid matrices. Analysis was conducted using thermal desorption/gas chromatography with flame photometric detection and thermal desorption/multidimensional gas chromatography mass spectrometry.

14. SUBJECT TERMS

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PREFACE

The work described in this report was authorized under Contract No. DAAM01-97-D-0005. This work was started in March 2000 and completed in July 2000.

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STIRBAR MICROEXTRACTION FOR DETECTION OF CW AGENTS IN LIQUID MATRICES

1. INTRODUCTION

Methods are needed for the trace analysis of chemical warfare materials in aqueous samples for several applications. These include verification of demilitarization processes, compliance with the Chemical Weapons Convention, monitoring of water supplies near chemical warfare storage sites, and cleanup of former production and testing sites.

Stirbar microextraction is a relatively new technique used to extract organic compounds from liquid matrices. It uses a glass stirbar encased with polydimethylsiloxane tubing (Figure 1). The stirbar is placed in the sample. The sample container is placed on a stirplate and the stirbar spun for a set period of time. The stirbar is then removed from the sample, dried, and placed in an empty thermal desorption tube. The tube is placed in a thermal desorption unit and analyzed by gas chromatography (GC).

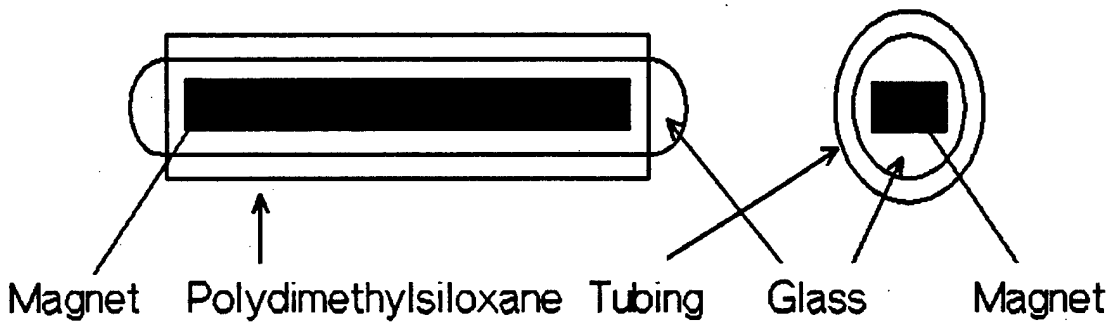

Figure 1. Microextraction Stirbar

The analytes chosen for this study were isopropyl methylphosphonofluoridate (GB), O-ethyl-S-(2-diisopropylaminoethyl)methyl phosphonothiolate (VX), and bis-(2-chloroethyl) sulfide (HD). Their structures are shown in Figure 2. These agents have varying solubilities in water. GB is miscible with water, VX is slightly soluble in water, and HD is barely soluble in water. A previous study[1] shows that the recovery of GB and VX from aqueous solutions is highly dependent on the pH. At pH <7, VX is protonated, making it harder to extract with organic solvent. At pH >7, GB rapidly hydrolyzes to isopropyl methylphosphonic acid.

[1]Durst, H.D. et al., *Report on the Analysis of Johnston Atoll Ton Container Decontamination Solution Samples*, Program Manager for Non-Stockpile Chemical Materiel, EAI Report Number A202/97/001F, EAI Corporation, Abingdon, MD, November 1997.

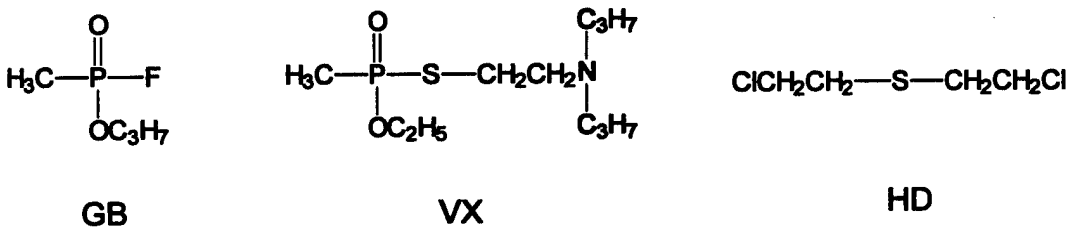

Figure 2. Chemical Structures for GB, VX, and HD

2. EXPERIMENTATION

The Twisters were placed in an empty desorption tube and thermally desorbed. The conditions used for the analyses are discussed in Sections 2.1 and 2.2.

2.1 Dynatherm/Agilent 5890 with Dual Flame Photometric Detector.

Oven: 50 °C (1 min), 15 °C/min to 245 °C
Detector: 240 °C

Dynatherm ACEM 900 Thermal Desorber
Tube: 225 °C (5 min, 35 mL/min flow)
Trap: 250 °C (3 min)
Transfer Line: 225 °C
Valve: 240 °C

2.2 Gerstel TDS2/Agilent 6890 GC/5973 MSD.

Thermal Desorption System:

Initial Temperature:	30 °C
Program Rate:	60 °C/min
Final Temperature:	250 °C
Final Time:	5 min

Cooled Inlet System:

Initial Temperature:	30 °C (-20 °C for GB)
Program Rate:	12 °C/sec
Final Temperature:	300 °C
Final Time:	5 min

Cryo Trapping System:

	GB	HD/VX
Initial Temperature:	-20 °C	-10 °C
Initial Time:	6.5 min	19.2 min
Program Rate:	0.5 °C/sec	12 °C/sec
Final Temperature:	160 °C	250 °C
Final Time:	6 min	13 min

GC Oven:

	GB	HD/VX
Initial Temperature:	50 °C	50 °C
Initial Time:	1.0 min	1.0 min
Program Rate 1:	15 °C/min	15 °C/min
Final Temperature:	110 °C	260 °C
Program Rate 2:	50 °C/min	50 °C/min
Final Temperature:	50 °C	50 °C
Program Rate 3:	15 °C/min	15 °C/min
Final Temperature:	155 °C	250 °C
Total Time:	13.2 min	32.5 min

Mass Spectrometer:

Selected Ion Mode:
GB (99 and 125 amu)
HD (109, 111, 158, and 160 amu)
VX (114, 127, 139, and 72 amu)

3. RESULTS

Initial experiments were carried out on a Dynatherm ACEM 900 thermal desorber attached to an Agilent 5890 GC with dual flame photometric detector (DFPD).

As a first experiment, the three agents were spiked in distilled, deionized water at a level of 1 ppb. Sample (20 mL) was pipetted into a 22-mL vial. A Twister was added and spun for 2 hr at room temperature. The Dynatherm/GC/DFPD was calibrated using equivalent amounts of the three agents, spiked onto a Tenax thermal desorption tube, as were spiked into the DI water samples. The Tenax tube was thermally desorbed using the same conditions that were used to desorb the Twisters. The results showed a 0.61% recovery for GB, 0.58% for VX, and 0% for HD.

The next experiment optimized the amount of potassium chloride (KCl) added to the sample to salt out the analytes to increase recovery. The amounts of KCl added were 0, 2, 4, and 8 g/20 mL of sample. The optimum amount of KCl was found to be 4 g/20 mL of sample.

Again, the samples were spiked to approximately 1 ppb. The recovery for GB increased from 0.61 to 1.33%. The recovery for HD increased from 0 to 40.8%. The recovery for VX increased from 0.55 to 1.22%. All samples were run for 2 hr at room temperature.

Next, the pH of the sample was varied, and the recoveries were calculated. The samples were spiked to 1 ppb and run for 2 hr. The recoveries for GB were 1.22% at pH = 5 and 0% at pH = 10. The recoveries for HD were 36.3 at pH = 5 and 36.0 at pH = 10. The recoveries for VX were 1.46% at pH = 5 and 14.2% at pH = 10. The results were consistent with the facts that GB rapidly hydrolyzes above pH = 7 and that VX is protonated below pH = 7.

A time study was conducted to determine the optimum time needed to stir the samples. Samples were spiked with HD/VX at a level of 1 ppb in DI water. The pH of the water was adjusted to 10 with 1.0 N NaOH. Four grams of KCl were added to each sample. For GB, samples were spiked at a level of 1 ppb. The pH was adjusted to 5 with 1.0 N HCl. Time points of 10, 30, 60, 90, 120, 180, and 240 min were used. The percent recoveries versus time are shown in Figures 3, 4, and 5 for GB, HD, and VX, respectively. The recoveries for the agents do not increase significantly after 1 hr of sampling.

Triplicate analyses were run on samples spiked at 1 ppb, pH = 5, 2 hr sampling, and 4 g KC1/20 mL of sample. The results showed good precision with recoveries for GB of 1.34, 1.30, and 1.34%. The recoveries for HD were 38.9, 42.1, and 41.5%. The recoveries for VX were 1.20, 1.26, and 1.20%. Five replicates were run for HD and VX at a spike level of 750 parts per trillion (ppt). The samples were 20-mL samples of water with the pH adjusted to 11.2 with 1.0 N NaOH. Four grams of KCl were added to each sample. The spin time for the extractions was 90 min. The average recovery for HD was 46.4% with a relative standard deviation (RSD) of 9.0%. The average recovery for VX was 18.0% with an RSD of 16%.

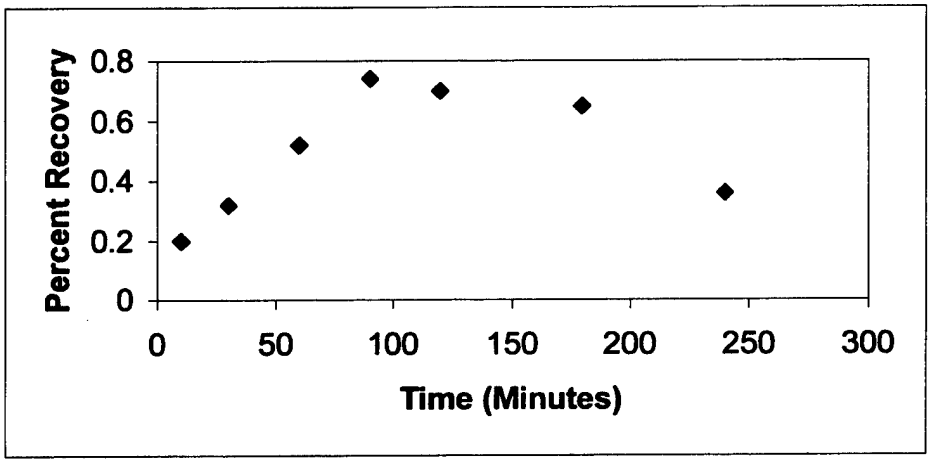

Figure 3. Percent Recovery Versus Spin Time for GB

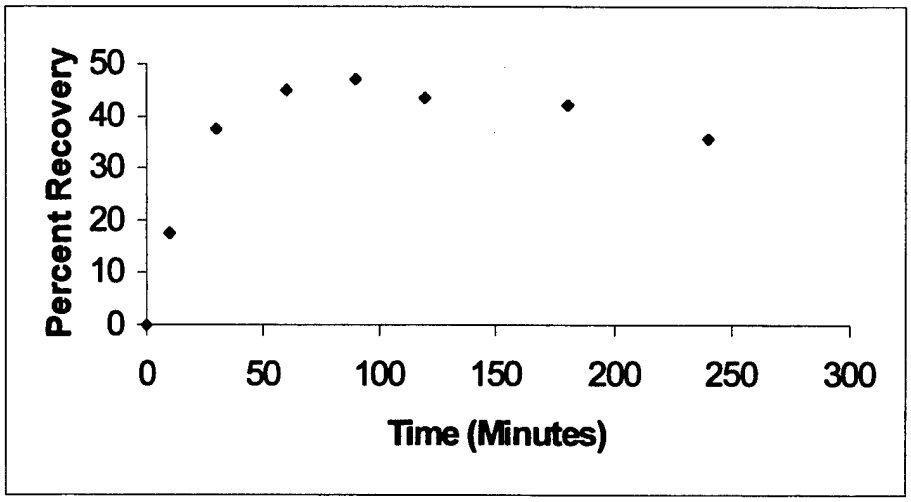

Figure 4. Percent Recovery Versus Spin Time for HD

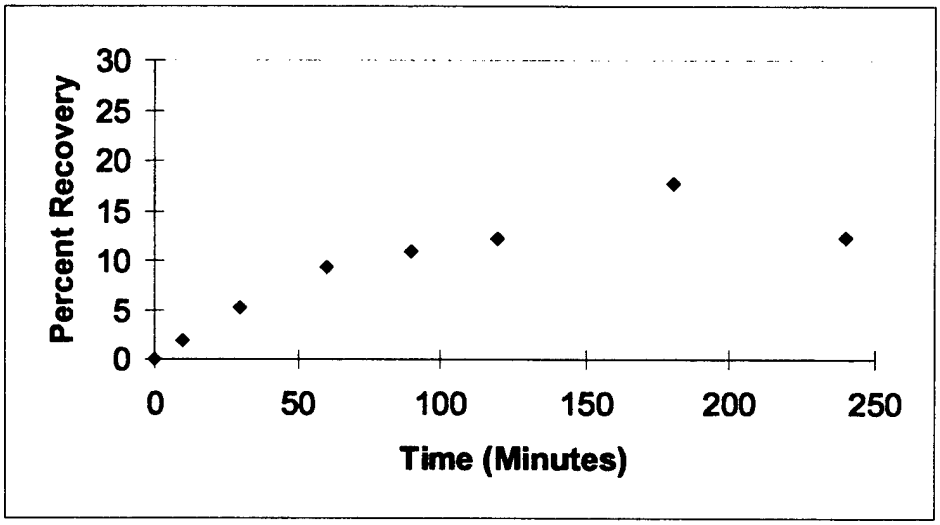

Figure 5. Percent Recovery Versus Spin Time for VX

Representative chromatograms are shown in Figures 6 and 7. Figure 6 shows an overlay of the P and S channels of the DFPD for all three agents in a 20-mL sample of water. The pH of the sample was 5, and 4 g of KC1 was added. The sample was stirred for 2 hr. Figure 7 shows HD and VX in a pH = 10 sample. It was also stirred for 2 hr, and 4 g of KC1 was added.

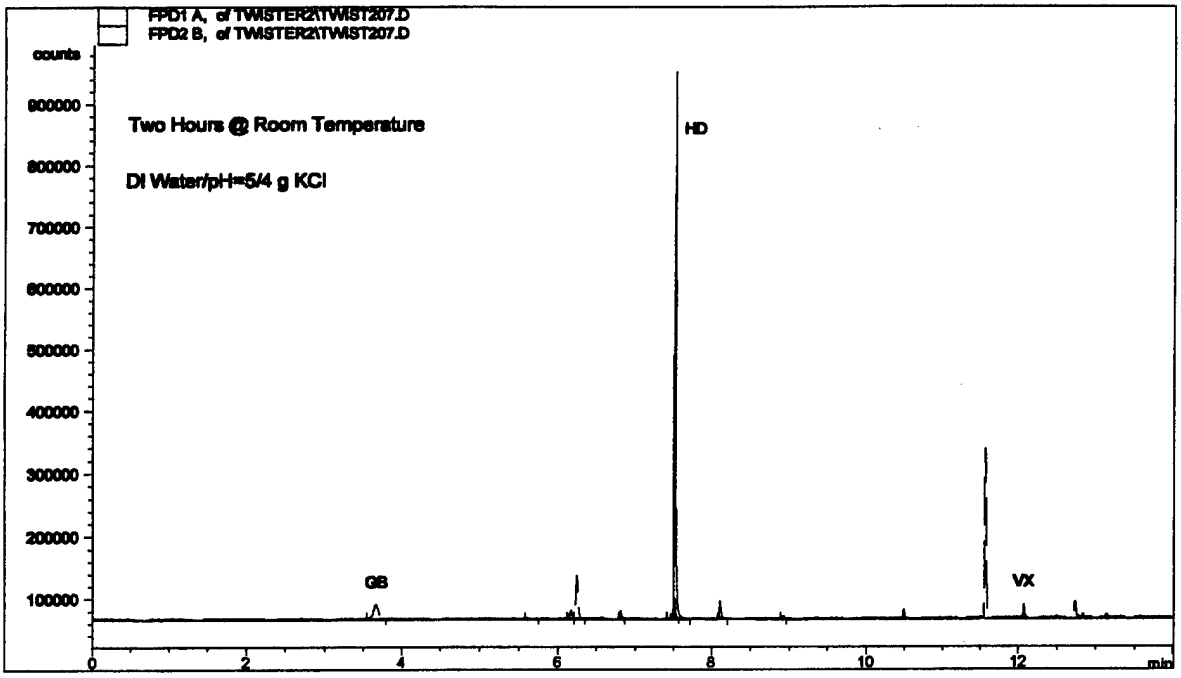

Figure 6. Overlay of P Channel for GB and VX, and S Channel for HD, for a pH = 5 Water Sample. The spike levels were 1.5 ppb for GB, 2.9 ppb for HD, and 1.4 ppb for VX.

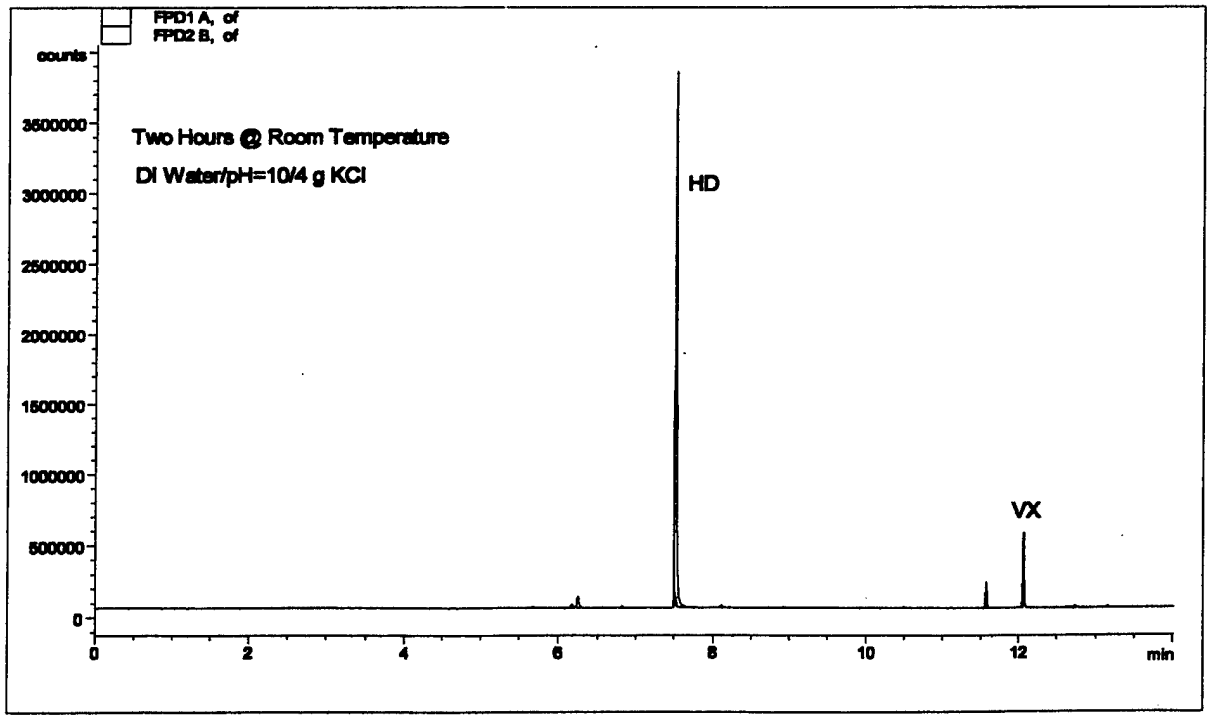

Figure 7. Overlay of P Channel for VX, and S Channel for HD, for a pH = 10 Water Sample. The spike levels were 2.9 ppb for HD and 1.4 ppb for VX.

Calibration curves for HD and VX were constructed by spiking samples in the range of 0 to 200 ppt. The samples were distilled water with the pH adjusted to 10.8 with 1.0 N NaOH. The sample volume was 20 mL to which 4 g of KCl was added. The spin time was 90 min. A solvent blank spiked with 5 µL of isopropanol (IPA) was analyzed in the same manner. Figure 8 shows the curve obtained for HD, and Figure 9 shows the curve obtained for VX. Both curves show excellent linearity in the 0 to 200 ppt range.

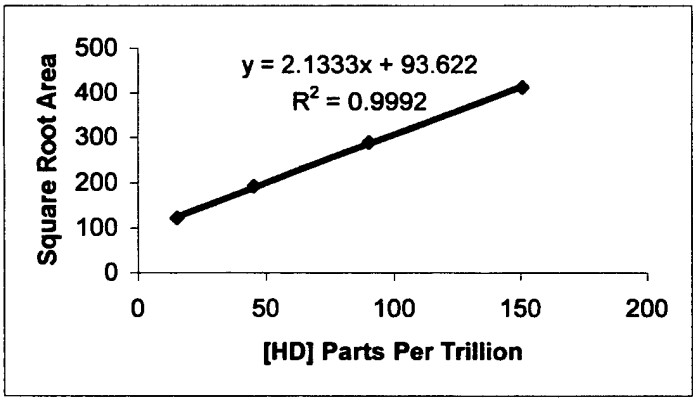

Figure 8. Calibration Curve for HD by GC/DFPD

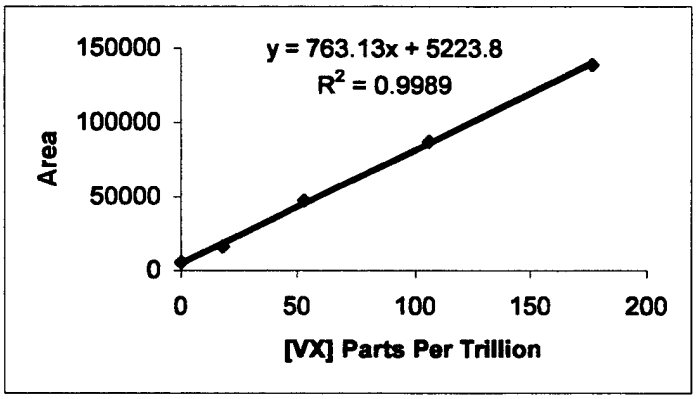

Figure 9. Calibration Curve for VX by GC/DFPD

The extraction of GB was optimized relative to the pH of the aqueous solution. Three 20-mL samples were acidified with 1.0 N HCl. The pHs used were 1.81, 3.06, and 5.58. The spin time for each sample was 90 min. Four grams of KCl was added to each sample. The relative peak areas for the three samples were 0.12:0.84:1.0 for the pH 1.81:3.06:5.58 samples, respectively. This shows the pH should be above 3 and below 7 for GB extraction.

At this point in the study, a Gerstel thermal desorber was purchased and used with an Agilent Model 6890 GC and Model 5973 Mass Selective Detector (MSD). The GC/MS system also consisted of a Gerstel Cooled Inlet System (CIS) and a Dual Column Switching (DCS2) for multidimensional chromatography. The optimized conditions for this configuration can be found in Section 2.2 of this report.

Calibration curves for HD and VX were constructed by spiking samples in the range of 0 to 200 ppt. The samples were distilled water with the pH adjusted to 10.8 with 1.0 N NaOH. The sample volume was 20 mL to which 4 g of KCl was added. The spin time was 90 min. A solvent blank, spiked with 5 µL of IPA, was analyzed in the same manner. Figure 10 shows the curve obtained for HD. Figure 11 shows the curve obtained for VX. Both curves show good linearity in the 0 to 200 ppt range.

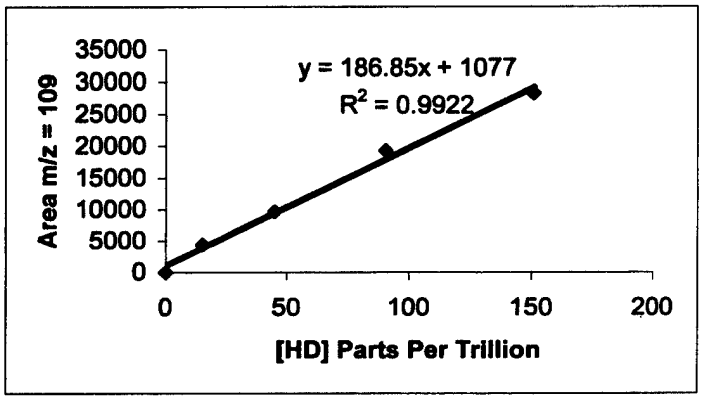

Figure 10. Calibration Curve for HD Using GC/MS

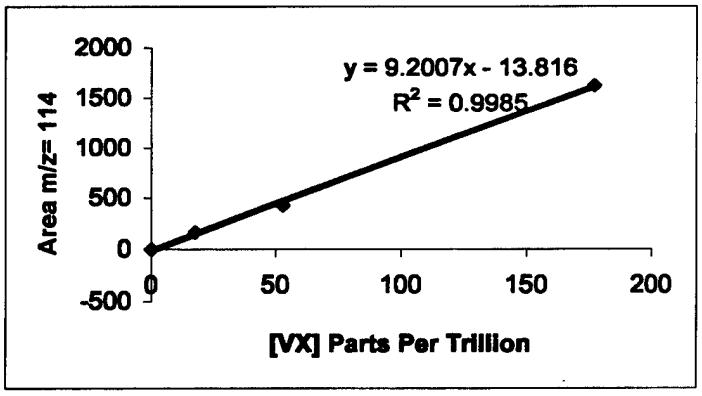

Figure 11. Calibration Curve for VX Using GC/MS

Calibration curves for GB in pH = 4 buffer and in local pond water were developed. Samples were 20 mL in volume. Four grams of KCl were added to all samples. The

pH of the pond water was adjusted to 4.4 with 1.0 N HCl. The spin time was 90 min. The resulting curves are shown in Figure 12. Both show good linearity in the range of 0-4 ppb. The correlation coefficient for GB was 0.99 in pH = 4 buffer and 0.98 in pond water.

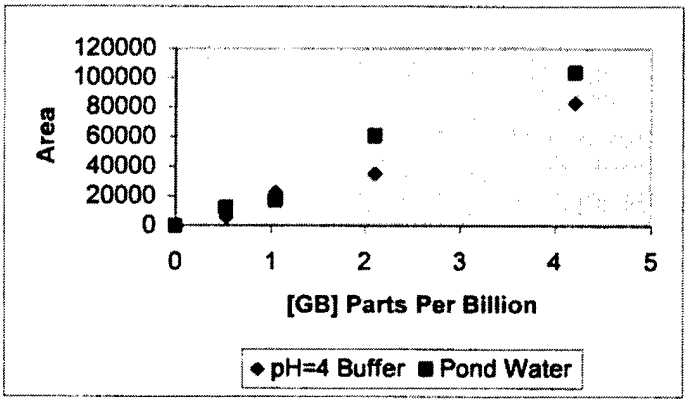

Figure 12. Calibration Curves for GB in pH = 4 Buffer and Pond Water Using GC/MS

4. APPLICATIONS

4.1 Pond Water.

A sample of local pond water was collected. The pH of the water was adjusted to 10.0 with 1.0 N NaOH. Four 20-mL samples were placed in 22 mL vials. Three of the samples were spiked with a standard of VX and HD. The fourth sample was used as a blank. The spike resulted in a level of 151 ppt of HD and 176 ppt of VX in the samples. A separate 20-mL sample of DI water was also spiked to the same level and analyzed. The samples were spun for 90 min. Four grams of KCl was added to each sample. The samples were quantitated against a similar amount of agent spiked onto a Tenax tube and thermally desorbed into the GC/MS. Neither VX nor HD were detected in the blank. The average recoveries from the pond water samples were 25.4% for HD and 4.38% for VX. The recoveries for the DI water were 28.5% for HD and 11.3% for VX. There appears to be some matrix effects, which lower the recovery of VX from the pond water. Even with the matrix effects, the VX is easily detected at a spike level of 176 ppt. Figure 13 shows the flame ionization detector (FID) trace for the pond water. The retention time for HD on the first column is 7.75 min. The retention time for VX is 12.8 min. Figures 14 and 15 show the total ion chromatograms in the retention windows for HD and VX, respectively.

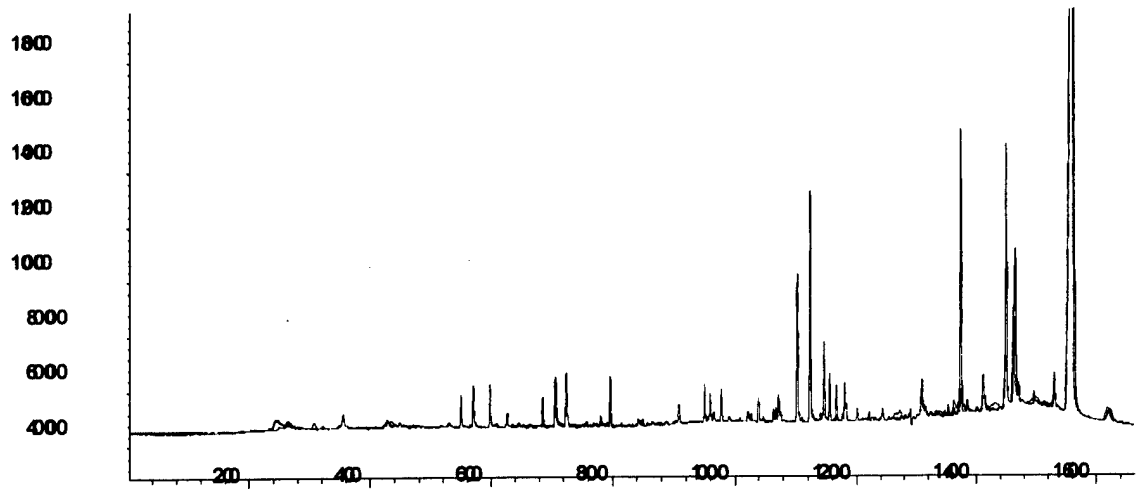

Figure 13. FID Trace of Local Pond Water

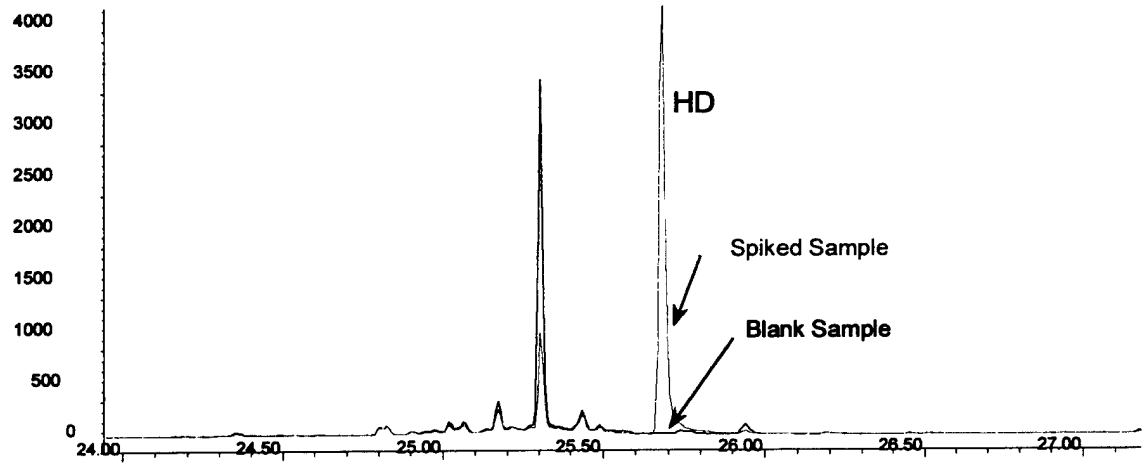

Figure 14. HD Cut of Pond Water, 151 ppt Spike

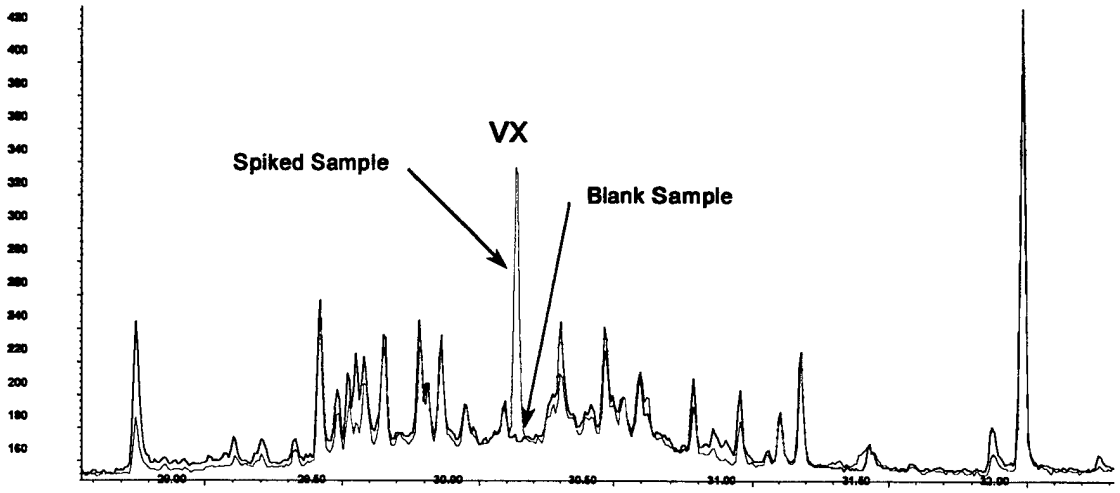

Figure 15. VX Cut of Pond Water, 176 ppt Spike

Stirbar microextraction was also successful in extracting GB from the pond water. The pH of the pond water was adjusted to 4.45 with 1.0 N HCl. Samples (20 mL) were spiked with GB in the 0.5-5 ppb range, and 4 g of KCl was added to each. The samples were extracted for 90 min. The GB cut area of the total ion chromatogram for four standards and a blank is shown in Figure 16.

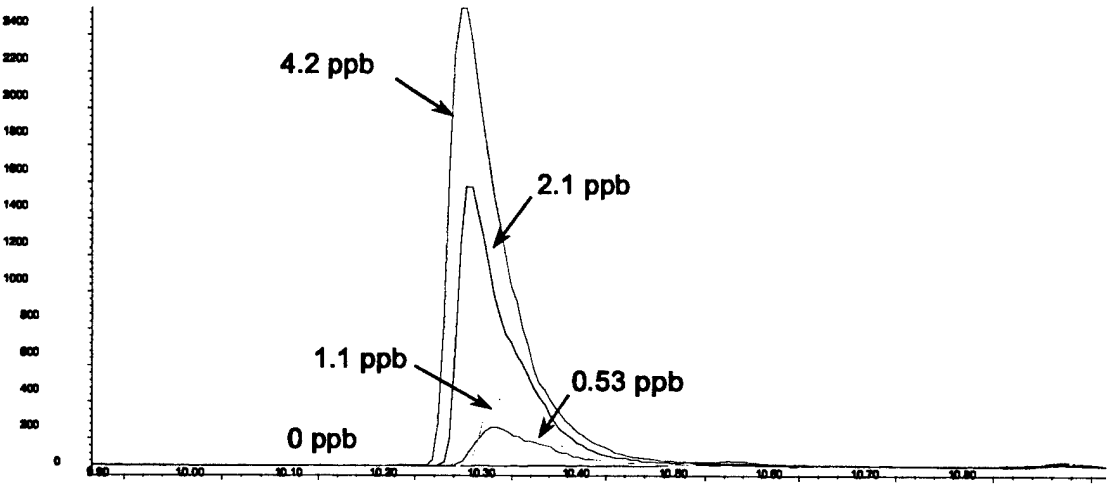

Figure 16. TICs of GB Standards in Pond Water

4.2 HD in Monoethanolamine (MEA)/NaOH Decontamination Solution.

Monoethanolamine/NaOH is used as a decontaminating reagent for GB, HD, and VX. Methods are needed for trace detection of these agents in the decontamination solution to verify destruction of the agent. Stirbar microextraction provides an attractive alternative to conventional liquid/liquid extraction for trace level detection of these agents in decontamination solution. A reaction mass from the decontamination of HD with MEA/NaOH was used to study the extraction of HD from these types of reaction masses. A 100 mg sample of reaction mass was spiked to 40 ng/g with HD. The spiked reaction mass was diluted to 20 mL with DI water. Four grams of KCl was added, and the mixture was extracted for 90 min with a microextraction stirbar. An unspiked reaction mass was prepared in a similar manner. Figure 17 shows the FID trace for the unspiked reaction mass. The retention time for HD on the first column is 7.7 min. Figure 18 shows the total ion chromatogram for the cut region of HD on the analytical column for the blank and spiked samples. The HD is clearly seen in the spiked sample. No HD appears in the blank sample.

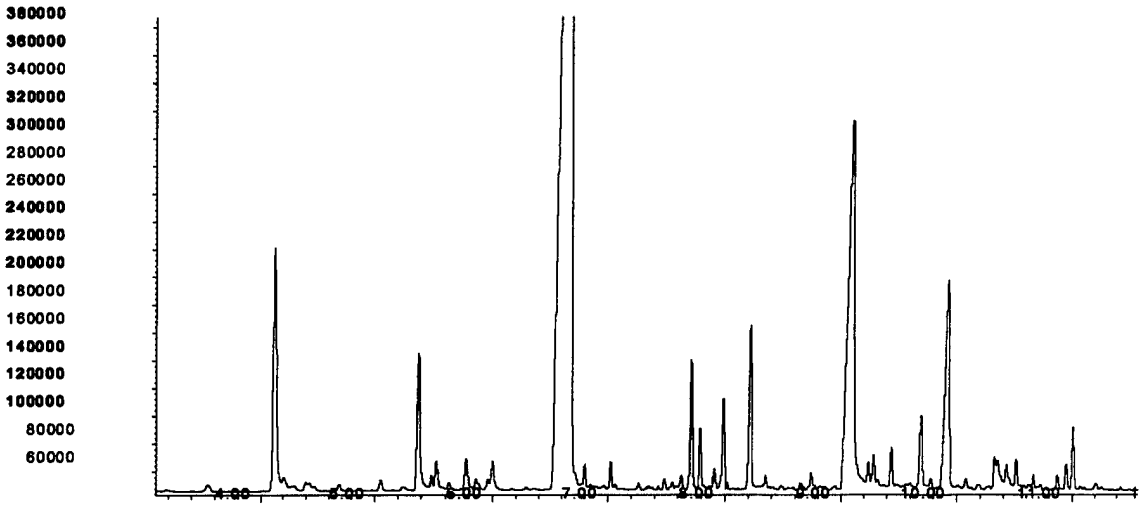

Figure 17. FID Trace of Unspiked HD/MEA/NaOH Reaction Mass

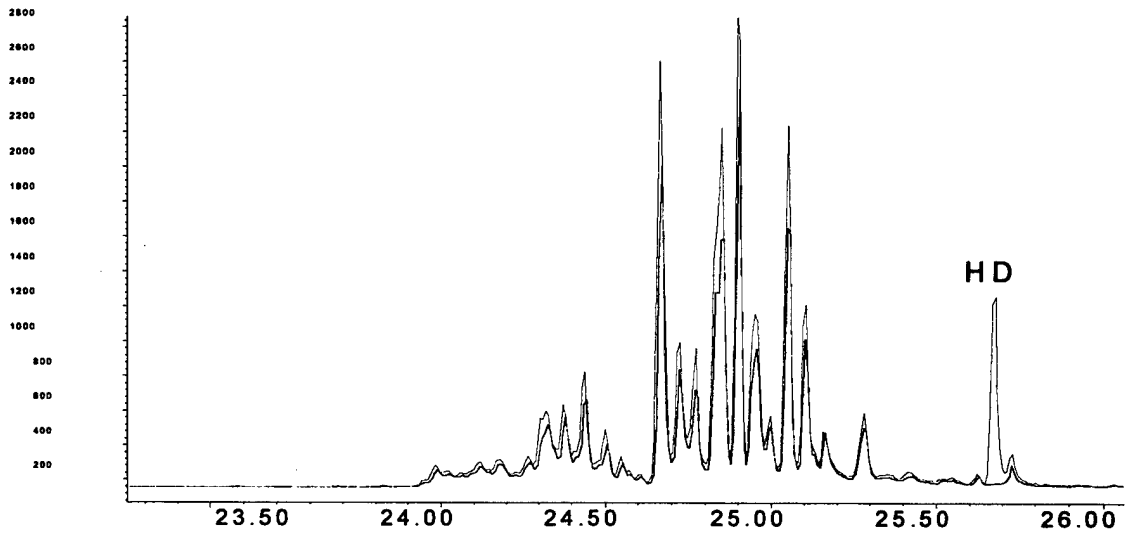

Figure 18. TICs of HD Cut Area from Spiked and Blank HD/MEA/NaOH Reaction Masses

4.3 GB and VX in Aqueous Bubbler Solutions.

An aqueous solution of tris(2-hydroxymethyl)amoniomethane was used as an air sampling media in bubblers for GB and VX in assessing technologies for destroying chemical agents. As an option to liquid/liquid extraction, stirbar microextraction was explored for detection of these agents in the bubbler solution. Samples (10 mL) were used for this study. Two grams of KCl were added to each sample. The pH of the buffer was adjusted to 3.5 with 1.0 N HCl for the analysis of GB. The pH = 8.3 buffer solution was not adjusted to analyze VX. A minimum detection limit (MDL) study was conducted for both agents in the bubbler solution. The MDL study consisted of five spikes at a mid-spike level and seven spikes at a low-spike level. Three matrix blanks and two DI water blanks were also run. The low- and mid-spike levels were 305 and 611 pg/mL, respectively, for GB. The low- and mid-spike levels were 31.1 and 62.2 pg/mL, respectively, for VX. An external calibration curve was generated for each agent by spiking a known amount on Tenax and thermally desorbing the tube into the GC using the same conditions as those used for the microextraction stirbars. Table 1 gives a statistical analysis of the data. Though the recoveries are low, the sensitivity and reproducibility of this technique are excellent for this application.

Table 1. Statistical Analysis of MDL Studies Data

Analyte	Spike Level (ppt)	Average Recovery (%)	Standard Deviation
GB	305	2.5	11.5
GB	611	1.7	14.4
VX	31.1	15	3.29
VX	62.2	10	10.2

4.4 GB, HD, and VX in Hydraulic Fluid.

Hydraulic fluid is used in pumps for decontamination reactors. Prior to disposal, used hydraulic fluid must be analyzed for chemical agent contamination. Stirbar microextraction was explored as an option to either liquid/liquid or solid phase microextraction[2] for analyzing GB, HD, and VX in hydraulic fluid. The fluid used was Mobil DTE 24. It is described as a hydrocarbon lubricant. The stirbar microextractors were placed into 4 mL of hydraulic fluid sample and spun for 90 min. The stirbars were removed from the hydraulic fluid, rinsed with methanol, and dried with a laboratory wipe. The samples were then analyzed by thermal desorption/multidimensional GC/MS. Samples were spiked with GB, HD, and VX. Figure 19 shows a total ion chromatogram for a GB spike at 100 ppb. Figure 20 shows a sample spiked at 250 ppb of HD. Figure 21 shows a total ion chromatogram for a hydraulic fluid sample spiked to a level of 10 ppm with VX. The results show that stirbar microextraction is a useful technique for extracting GB and HD from this complex hydrocarbon matrix. The VX was not easily extracted from the matrix but could be detected at a 10 ppm level.

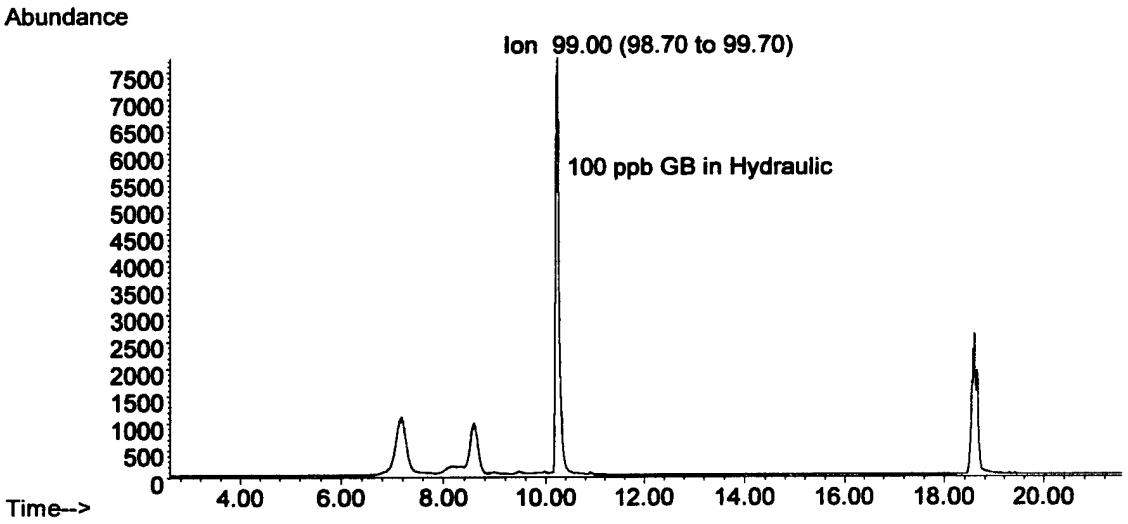

Figure 19. GB in Hydraulic Fluid

[2]Stuff, J.R., Cheicante, R.L., Creasy, W.R., Connell, T., Ruth, J.L., Heykamp, L.S., Rodriguez, A.A., and McGarvey, D.J., *Development and Evaluation of Analytical Methods to Support PMNSCM Field Testing Demo*, Program Manager for Non-Stockpile Chemical Materiel, EAI Report Number 400006.014/99/001F, EAI Corporation, Abingdon, MD, October 1999.

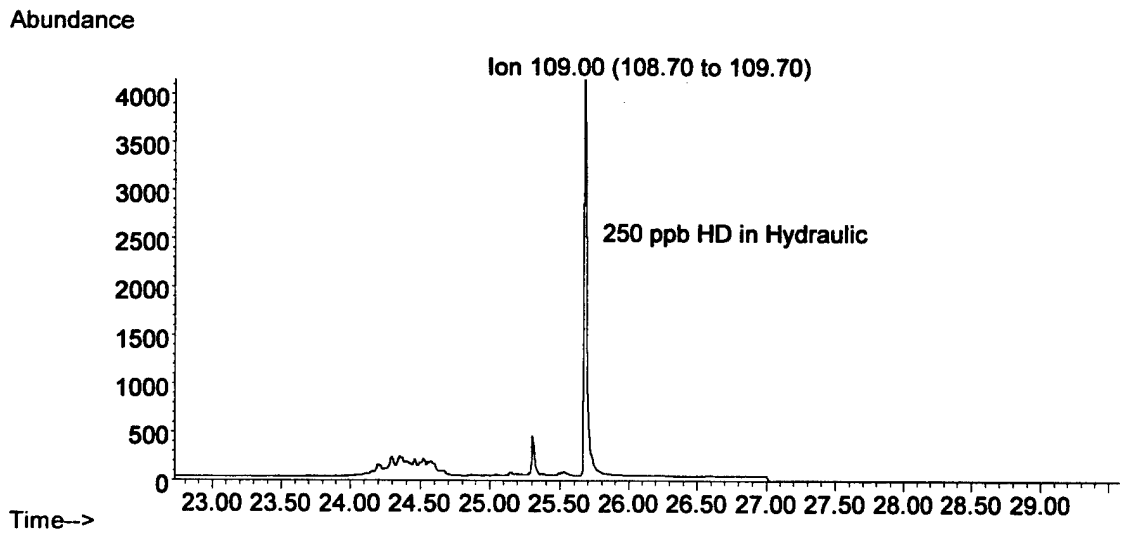

Figure 20. HD in Hydraulic Fluid

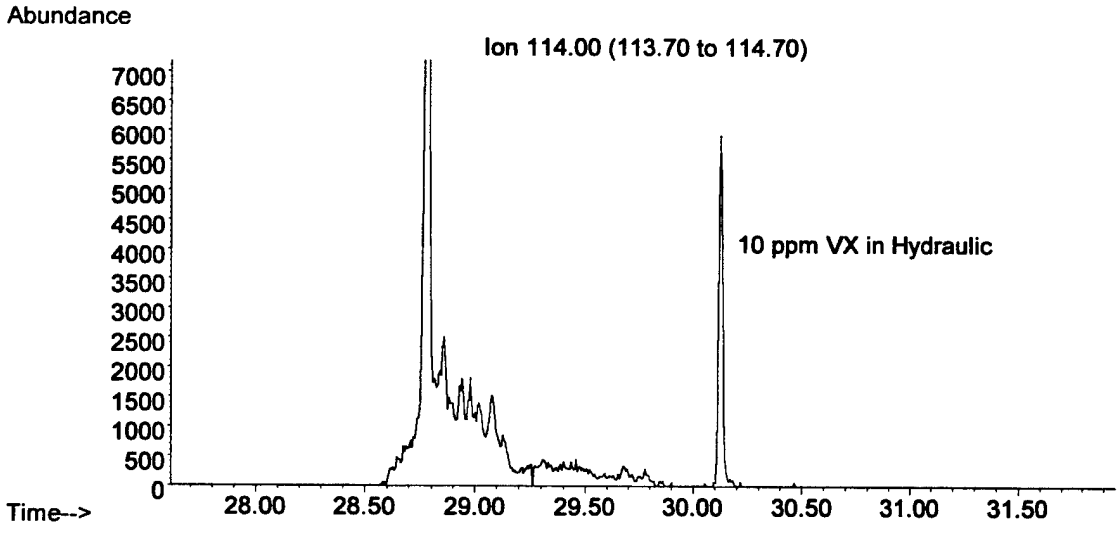

Figure 21. VX in Hydraulic Fluid

5. CONCLUSIONS

Stirbar microextraction is an effective technique for extracting GB, HD, and VX from aqueous-based matrices. This technique negates the use of hazardous organic solvents and subsequent steps to concentrate the extracts. It is a low-cost, easy to use alternative to liquid/liquid extraction. The stirbars used in this study were reused over 50 times with no apparent degradation in performance. The technique is very amenable to field use. The detection limits for the agents in water are shown in Table 2. The stirbar microextraction technique can also be used on non-aqueous matrices as shown by the extraction of the agents from hydraulic fluid. Stirbar microextraction in combination with multidimensional gas chromatography/mass spectrometry is an excellent approach to the analysis of complex mixtures.

Table 2. Detection Limits for Agents by MS and DFPD

Selected Ion Mass Spectrometry		
Agent	Concentration (ppt)	S/N Ratio
GB	530	25:1
HD	15.1	51:1
VX	17.7	5:1
Flame Photometric Detection		
Agent	Concentration (ppt)	S/N Ratio
GB	1500	7:1
HD	15.1	2:1
VX	17.7	6:1
20 mL samples, 4 g KCl, 90-min spin time, pH = 4-5 (GB), 10 (HD/VX)		

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